priority of provisional application Serial No. 60/096,938, filed August 20, 1998, entitled "The 5' Untranslated Region of the Cold-Shock *cspA* Gene Regulates Translation Efficiency in Addition to mRNA Stability," and U.S. provisional application Serial No. 60/143,380, entitled "Translational Enhancement by an element Downstream of the Initiation Codon in *Escherichia coli*."

Marked-up Verison in the Claims

1. (Twice Amended) An isolated ~~ribo~~nucleic acid molecule that ~~prolongs~~ regulates the expression of a cold shock inducible gene under physiological conditions ~~that elicit~~which cause the cold shock response in a bacterium, wherein said isolated nucleic acid molecule is mediated by a portion of a 5'-UTR of the cold shock inducible gene or a substantially homologous sequence thereof.

3. (Twice Amended) The isolated ~~ribo~~nucleic acid molecule of Claim ~~2~~1, wherein said 5'-UTR is a 5'-UTR of a cold-shock inducible gene selected from the group consisting of *cspA, cspB* and *csdA.*

5. (Twice Amended) The ~~ribo~~nucleic acid molecule of Claim 3, wherein said 5'-UTR comprises nucleotides +1 to +11 of the *cspA* 5'-UTR (nucleotides 1 to 11 of SEQ. ID. NO. 55) or a nucleotide sequence having substantial homology to nucleotides +1 to +11 of the *cspA* 5'-UTR (nucleotides 1 to 11 of SEQ. ID NO. 55).

6. (Twice Amended) The isolated ~~ribo~~nucleic acid molecule of Claim1, wherein said cold shock inducible gene interacts with CspA protein.

7. (Twice Amended) An isolated ~~ribo~~nucleic acid molecule that represses the expression of a cold shock inducible gene under physiological conditions.

8. (Twice Amended) The isolated ~~ribo~~nucleic acid molecule of Claim 7, comprising at least a portion of the 5'-UTR of a cold shock inducible gene.

9. (Twice Amended) The isolated ~~ribo~~nucleic acid molecule of Claim 8, wherein said cold-shock inducible gene is selected from the group consisting of *cspA, cspB, and csdA.*

11. (Twice Amended) A non-coding ~~ribo~~nucleic acid molecule that enhances the translation of a cold shock inducible gene under conditions that elicit the cold shock response of a bacterium.

12. (Twice Amended) The ~~ribo~~nucleic acid molecule of Claim 11 comprising at least a portion of the 5'-UTR of a cold shock inducible gene.

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13. (Twice Amended) ~~ribo~~The nucleic acid molecule of Claim 12 wherein said cold shock inducible gene is selected from the group consisting of *cspA, cspB,* and *csdA*.

14. (Thrice Amended) The ~~ribo~~nucleic acid molecule of Claim 13, comprising nucleotides +123 to +135 of the *cspA* 5'-UTR (nucleotides 123 to 135 of SEQ. ID. NO. 55) or a nucleotide sequence having substantial homology to nucleotides +123 to +135 of the *cspA* 5'-UTR (nucleotides 123 to 135 of SEQ. ID. NO. 55).

15. (Twice Amended) The ~~ribo~~nucleic acid molecule of Claim 14 comprising a sequence selected from the group consisting of SEQ ID NO:48, SEQ ID NO:49, and SEQ ID NO:50.

Please cancel Claims 2 and 4 without prejudice or without disclaimer of the subject matter contained therein.

Please add the following new Claim 57:

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57. (New) An isolated nucleic acid molecule component to prolong the expression of a cold shock gene during abdendation of a bacterium to physiological stress which elicits a cold shock response, said nucleic acid molecule comprising at least 8 of the first 25 nuclear tag of a 5'-UTR with a cold shock inducible mRNA transcript, and a promoter active under conditions of physiological stress to induce said cold shock response in said bacterium.

REMARKS

Priority

Applicants acknowledge the Examiner's objections to the priority data contained in page 1 of the amended specification, and as a result the Applicants have amended the first paragraph of page 1 of the specification to reflect the proper priority data.

Claim Rejections – 35 U.S.C. § 112

The Examiner has rejected Claims 1-4, 6-9, 11-13, 16-56 under 35 U.S.C. § 112, first paragraph, as containing subject matter, which was not described in the specification in such a way so as to convey to one skilled in the relevant art that the inventor was in possession of the claimed invention at the time the application was filed. Applicants respectfully traverse this rejection and submit that the claims, as amended, along with the detailed specification, offer ample support to show that the Applicants were in possession of the claimed invention at the time the application was filed.

Applicants respectfully submit that the Federal Circuit has consistently held that the requirements of section 112 are met despite the need for some experimentation by those skilled in the art. Specifically, we invite the Examiner's attention to U.S.S. v. Tele Electronics, Inc., wherein the court held that the "test for enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent, coupled with information known in the art, without undue experimentation." U.S.S. v. Tele Electronics, Inc., 857 F.2d 778, 8 U.S.P.Q.2d 1217 (Fed. Cir. 1998). The Applicants respectfully submit that the test for enablement is not whether further experimentation would be required, as helpfully suggested on page 3 of the November 19, 2002 Office Action, but rather the test is whether "undue"

experimentation would be required by those skilled in the art. In view of the foregoing, Applicants respectfully submit that undue experimentation is not required to identify sequences which are substantially homologous to the well known and well described sequences of *cspA, cspB,* and *csdA* genes. Applicants further respectfully submit that undue explanation is not requested to identify which of the substantially homologous sequences to *cspA* function as a cold shock inducible gene. The Applicants invite the Examiner's attention to page 2 of the Applicants specification wherein the *cspA* gene of fully mapped *E. coli* is thoroughly described in terms of both functionality and structure. Specifically, *cspA* consists of 70 amino acid residues which have been identified as the major cold shock protein. Moreover *cspA* three-dimensional structure has been determined, and it was shown that *cspA* consists of five-antiparallel β-stranded structures.

Notably, at the time the Application was filed there were more than 50 proteins homologous to *cspA,* which had been identified in a variety of prokaryotes. As an example of the knowledge in the art, we invite the Examiner's attention to Yamanaka et al., which describes nine genes encoding *cspA* like proteins. These proteins and their role as cold shock proteins have been identified and characterized (Yamanaka, K., L. Fang, M. Inouye 1998, The *cspA* family in *E. coli.*: Multiple gene duplication for stress adaptation. Mol. Microbiol. 27:247-255). Furthermore, we invite the Examiner's attention to Goldstein et al., wherein the 1990 reference acknowledged "Among several cold-induced proteins previously detected is one designated F10.6(g)." (Goldstein et al. 1990, pg. 286, col. 2).

Applicants respectfully submit that given the detailed knowledge of the "mapped" *E. coli.* genenome, along with very well characterized cold shock genes, one skilled in the art could readily find substantially homologous sequences of *cspA* and test the sequences cold shock

expression and subsequent mediation through the guidelines described in the Applicants' specification. In view of the well understood nature of cold shock inducible genes and in further light of the extensive examples, 13 of which the Applicants have provided, it is respectfully submitted that the Applicants clearly had possession of the claimed genus at the time the application was filed. Thirteen examples is a significant disclosure providing more than ample guidance to those of ordinary skill in the art.

Applicants respectfully submit that the pending application presents a similar factual scenario to *In re Angstadt,* 190 U.S.P.Q. 214 (C.C.P.A. 1976), wherein the Court squarely established that §112 does not require the disclosure of a test with every species covered by a claim. We invite the Examiner's attention to the following passage from *In re Angstadt:*

> Appellants have apparently not disclosed every catalyst which will work; they have apparently disclosed every catalyst which will not work. The question, then, is whether in an unpredictable art, section 112 requires disclosure of a test with every species covered by the claim. To require such complete disclosure would apparently necessitate a patent application or applications with "thousands" of examples with disclosures of "thousands" of catalyst along with information as to whether each exhibits catalyst behavior resulting from the over production of hydroperoxides. More importantly, such a requirement would force an inventor seeking adequate patent protection to carry out a prohibitive number of actual experiments. This would tend to discourage inventors from filing patent applications in an unpredictable area since the patent claims would have to be limited to those embodiments which are expressly disclosed. A potential infringer could readily avoid "literal" infringement of such claims by merely finding another analogous catalyst complex which would be used in "forming hydroperoxides.

At the time the Application was filed there were more than 50 proteins homologous *cspA* which had been identified in a large variety of prokaryotes. Moreover, a region called "cold shock domain" of a eukaryotic Y-box protein family, such as the human YB1 and zinopus FRGY[-2]

shared more than 40% identity with E. coli *cspA* (Wolfe et al. 1992), indicating that the cold shock domain was well conserved throughout evolution and organisms.

Applicants readily acknowledge that the art described in the specification is not totally predictable. However, given the vast knowledge of the *E. coli* genome, the prior art describing over 50 homologous *cspA* proteins, the well-described and identified *cspA* "like proteins" and the detailed description and examples throughout the Applicants' specification, it would seem redundant to have the Applicants repeat the examples as set out in the specification for all homologous sequences of *cspA*.

Response to 102 Rejections

Claims 1-15 are rejected under 35 U.S.C. § 102(b) as being anticipated by Goldstein et al. Applicant's respectfully traverse this rejection, and submit that in view of the claim amendments, and in further view of the reasons set forth below, the rejection is now obviated.

Anticipation requires that the prior art reference teach each and every element of the claimed invention. Applicants submit that Goldstein et al. provided a starting point for the characterization of the *cspA* gene. Specifically, the Examiner's attention is invited to column 1, page 283 of Goldstein et al., wherein it is clear that they teach the initial identification and sequencing of *cspA* gene. Goldstein et al. provides preliminary results indicating that the *cspA* gene may play role in protecting cells from damage due to freezing, as shown from the hyper expression of the protein coded for by *cspA* during a cold shock induction of 10°C. Nothing in Goldstein et al. indicates the regulation, induction and control of the *cspA* gene. Specifically, the Examiner's attention is invited to page 287, column 1, wherein Goldstein et al. teach that "obviously, much work needs to be done to elucidate the mode of regulation of the *cspA* gene as

well as the function of the CS7.4 protein." The Examiner is further asked to consider the following passage from Goldstein et al., which illustrates the time transient expression of the cold shock protein:

> As shown in Fig. 2B, induction was transitory with both the time of maximal induction and the maximal rate of synthesis dependent on the temperature.

In fact, prior to the Applicants' discovery, people skilled in the art, following the teachings in Goldstein et al., thought that the protein encoded for by *cspA* was capable of expression only so long as the cell was maintained in cold conditions. Specifically, the Examiner's attention is invited to page 286, column 2 of Goldstein:

> CS7.4 is unique among *E. coli.* proteins that exhibit increased synthesis in the cold in that is not detected at normal growth temperatures (30°C or 37°C; see ref. 8). Nevertheless, at its maximum rate, shortly after temperature shift, CS7.4 becomes the most abundantly synthesized protein in the cell. The stability of the protein and the fact that the protein continues to be synthesized after the initial burst of induction at 15°C indicate that a constant level of the protein is probably maintained in the cell as long as it remains exposed to cold temperatures. Although the protein does not appear to be actively degraded upon return to 37°C, the level of the protein is probably rapidly lowered by cell division in the absence of further synthesis.

As a result, it is clear that at time of Goldstein et al. the function of the 5'-UTR was not known, and as a result, the regulation of cold shock inducible genes was not well understood or characterized in the art. The Applicants, however, surprisingly discovered that the regulation of genes, expressed under conditions of physiological stress to elicit the cold shock response of bacterium, are mediated by 5'-UTR elements. Nothing in the prior art has disclosed the region or the role of the 5'-UTR in the mediation and regulation cold shock expression.

Turning to the rejection of claims under 35 U.S.C. § 102(b) as being anticipated by Oppenheim et al. (U.S. Patent No. 5,726,039) or Oppenheim et al. (U.S. Patent No. 5,654,169),

the Applicants respectfully traverse this rejection. Applicants respectfully submit that in view of the amendments to Claim 1, and in further view of the remarks set forth below, the rejection is now obviated.

Applicants respectfully submit that nowhere in either of the Oppenheim et al. patents, and more specifically, nowhere within column 13, lines 5-20 of '169; or column 14, lines 40-63 of '039, is there a discussion or teaching regarding the role of 5'-UTR. Specifically, the Examiner's attention is invited to the brief description of Fig. 7 and Fig. 10 in the '039 patent which illustrates that "EL" and "M" are promoters used to drive gene expression at low temperatures. Applicants respectfully submit that a promoter is a cite on DNA to which RNA polymers bind to initiate a transcription, whereas 5'-UTR is a region located at the 5' end of a mature transcript which preceeds an initiation code (promoter) and is not translated into a protein. It is well known in the art that 5'-UTR serves to regulate expression of genes located downstream of 5'-UTR. Moreover, nowhere in either of the Oppenheim et al. patents is there a discussion of the functional domains of 5'-UTR and its resulting effect on the mediation and expression of cold shock inducible genes. Further, there is no suggestion or teaching in Oppenheim et al. that the promoters discussed in Oppenheim et al. serve to prolong the normally transient expression of cold shock genes beyond the point in which physiological stress is removed. Specifically, we invite the Examiner's attention to column 14, lines 60-64 of the '039 patent wherein it is demonstrated that the "EL" and "M" promoters may be used to drive expression only at low temperatures. The Applicants, however, have illustrated expression well after the temperature is returned to normal. Thus, among other embodiments, the Applicants illustrate an extended expression period which can last beyond the removal or prior to the

position, of a physiological stress. As a result, Oppenheim fails to illustrate the controlled regulation of cold shock inducible genes through the use of 5'-UTR regions.

In light of the foregoing, Applicants respectfully submit that the specification and claims as amended are now in condition for allowance, which action is respectfully requested.

Respectfully submitted,

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